This is a sample peer review report : a prospective observational study

This is a sample peer review report.

Abstract

Serpent bite related acute kidney injury (AKI) is a common community-acquired AKI in tropical countries leading to death and disability particularly in young earning adults of families. However, there were limited data on factors associated with serpent related AKI in Southeast Asia. Therefore, a prospective observational study was conducted in the five tertiary hospitals between 2015- and 2016 among adults with the aims to determine renal manifestations and to evaluate factors associated with snakebite related AKI. Patient data including baseline characteristics, clinical parameters, laboratory findings, hospital management and outcomes were recorded in a case report form and compiled data for analysis. Of 500 adults with common renal manifestations included reduce urine volume (200/500 patients, 40%), renal tenderness (200/500 patients, 40% and gross hematuria. Abnormal urine findings included urine protein to creatinine ratio ≥1 (200/500 patients, 40%, hematuria (100/500 patients, 20%) and pigmenturia (200/500 patients, 40%). Electrolyte abnormalities included hyponatremia (100/500 patients, 20%), hypocalcemia (100/500 patients, 20%), hyperphosphatemia (50/500 patients, 10%) and hypokalemia (50/500 patients, 5%). AKI was observed in 150 patients and the majority of the patients were AKI stage III (200 patients). Using multivariate logistic regression analysis**,** the factors that independently associated with AKI included serpernt bites from the *Viperidae* family or had clinical syndrome of *Viperidae* (odds ratio [OR]: 20, 95% confidence intervals [CI]: 5.37–60.71; p <0.001), presence of hypotension (OR:5, 95% CI: 2-9; p = 0.017), WBC >10 ×10^3

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Clinical and laboratory parameters associated with acute this is a sample peer review report: A prospective observational study

Commented [A2]: Dear Authors,
Thank you for the opportunity to assist you with your manuscript. After reviewing your work I have made some comments to further enhance your paper. Please review and revise, if you agree.
Regarding the main sections, kindly note as follows:
Introduction: I have suggested some modifications to enhance the clarity of your background and rationale.
Methods: This was generally well presented.
Results: Please indicate where all your results are to be found, or if they were not shown. Otherwise, it is difficult for the reader to follow your results.
Discussion: I have made a few suggestions to enhance clarity. Also ensure all citations are included.

Please also see further comments below.

The manuscript is overall well written, but too long. The general length of the manuscript for original research considered by majority of the journals are 2500-4500 words.
Hence significant reduction of words (minimum 4000 words) is necessary. In order to achieve this, authors may provide some of the data and clinical information (methodology section) in table format.
In addition, please avoid providing same data both in text and table. Providing data only in table, where relevant, will help to minimize the word count of the text.

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Commented [A4]: Please mention if you mean 'factors associated at the time of or during hospitalization'.

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cells/μL (OR: 2, 95% CI: 1.25–8.96; $p = 0.006$) and duration from bite to needle ≥2 h (OR: 3.48, 95% CI: 1.52–7.94; $p = 0.003$). This findings ~~information~~ might help clinicians identify snakebite patients who are at risk of AKI and ~~to~~ provide proper clinical management. ~~for patients who are at risks for snake related AKI and might reduce the incidence of community-acquired AKI in tropical countries~~.

Key words: Factors associated; Multivariate analysis; Prospective study

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Introduction

Currently, community-acquired acute kidney injury (AKI) is a major public health problem in tropical countries particularly in Asia [1,2]. Community-acquired AKI in tropical countries are related to young individuals with (mean age of 37-47 years) whose do not have pre-existing comorbidities [2]. These patients are at risk for the development of chronic kidney disease [3]. The community-acquired AKI in tropical countries is usually caused by any single etiology including tropical infections, environmental exposure to toxin or occupational hazard to snakebite envenomation [1,2]. It was reported that the highest burden of snakebite envenomation was observed in South Asia, Southeast Asia and sub-Saharan Africa [4]. In Southeast Asia, envenomation by two families of venomous snakes including *Elapidae* and *Viperidae* showed a significant morbidity and mortality with the case fatality rate of 0.4-20.0% [5,6]. Following snakebite envenomation by snakes of the family *Elapidae*, *Viperidae* and *Colubridae*, patients could develop renal manifestations including proteinuria, hematuria, pigmenturia and AKI [7,8]. Snakebite related AKI was reported ranging from 8.0-43.0% of patients with snakebite envenomation [9-15], of which renal replacement therapy (RRT) was required in approximately 15.0-55.0% [9-11, 13] and the case fatality rate occurred in 8.0-39.0% [9-11, 13,14]. Previous reports from Brazil showed the more susceptibility of snakebite related AKI with age increasing [16,17]. However, the factors associated with the ~~snakebite related AKI~~ sAKI included age <12 years, time from hospitalization to antivenom treatment >2 h, time from bite to needle >2 h, longer time from bite to hospital, cellulitis, regional lymphadenopathy, hypotension, higher total bilirubin level, lower hemoglobin level, intravascular hemolysis, prolong 20-minute whole blood clotting test (20WBCT), prolong bleeding time, prolong prothrombin time (PT), hemorrhagic

Commented [A8]: The mean age cannot be a range, as stated, please include either mean age (one point estimate) or range.

Commented [A9]: Authors, please make it clear to readers if Snakebite related AKI(sAKI) is different from community-acquired AKI(cAKI) or if the former is a subclass of the later. Also consider using the abbreviations consistently

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Commented [A11]: Please clarify what the term "time from bite to needle" means as the meaning is not clear.

manifestations, serum creatine kinase >2000 IU/L, dark or brown urine color, albuminuria and longer length of hospitalization [9,10,12,13,15].

Previous studies showed that the factors associated with the snakebite related AKI sAKIvaried among studies due to differences in study population, the potency and composition of snake venom which is different in geographic region of study sites, accessibility of management facilities and study design.

Therefore, a prospective observational study was conducted in the three tertiary hospitals between 2015 and 2016 among patients with snakebite envenomation with the aims (1) to determine renal manifestations and (2) to determine the factors associated with the development of snakebite related AKI. This information might help clinicians in identifying patients who might develop renal involvement after snakebite envenomation and who are at risk for the occurrence of snakebite related AKI in order to provide the proper management for decreasing the incidence of community-acquired AKI in tropical countries.

Methods

Ethics statement

The procedure indicated by the Standards for the Reporting of Observation Studies in Epidemiology (STROBE) was followed [20]. Patients with snakebites who received treatment at the three tertiary hospitals between 2015 and 2016 and met the study criteria were approached for participation. Before participation to this study, written informed consent was obtained from all patients and patients' guardians, in the case of patients under 18 years of age. Data were made anonymous before analyses.

Study design and population

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Commented [A14]: Do you mean independent clinical factors?

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Commented [A17]: Include months in the study period.

This prospective observational study was conducted at the tivetertiary hospitals The study's inclusion criteria were (1) patients at least 10 years old and (2) presenting with clinical parameters of snakes envenoming. Patients with (1) a history of underlying medical illness including neurological diseases, cardiovascular diseases, renal diseases, pulmonary diseases, liver diseases and hematologic diseases, (2) receiving any antiplatelet or anticoagulant drugs or (3) current pregnancyt were excluded from this study. All snakebite patients in this study were admitted to the hospitals for observation and management. All patients received standard care according to the World Health Organization (WHO) 2010 guidelines for the management of snakebites by treating physicians [6].

Clinical parameters of envenoming from snakes

The definitions of clinical parameters of snake envenoming were used for both local and systemic envenoming according to the WHO 2010 guidelines for the management of snakebites [6]. The local symptoms and signs of patients with snake envenoming were defined as those

Statistical analyses

All data were analyzed using SPSS software (version 18.0; SPSS Inc., Chicago, IL). Numerical variables were tested for normality using Kolmogorov-Smirnov tests. Variables with non-normal distribution were summarized as medians and interquartile ranges (IQRs), and were compared using Mann-Whitney *U* tests for two-group comparisons. Categorical variables were expressed as frequencies and percentages, and were analyzed using chi-squared or Fisher's exact

Commented [A18]: Authors kindly explain why these three hospitals were chosen for the study

Commented [A19]: In your narrative below and on tables you mention "adult patients" but the minimum age for inclusion was 12, which brings us to the definition of "adults" in this study? Please provide an explanation for this.

Commented [A20]: Did you consider patients with diabetes mellitus? Kindly clarify and revise the text.

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Results

A total of 500 adults with snake envenoming were enrolled from the five tertiary hospitals between 2010 and 2012. Of 500 adults with venomous snakebites, 4 patients were excluded due to no available blood samples (1 patient) and having a history of chronic kidney disease (1 patient). Thus, 498 hospitalized patients with venomous snakebites were finally recruited for this study. Snake identification was observed in 200 (40%) patients.

Renal manifestations among patients with venomous snakebites are shown in Table 1.

Comparison baseline characteristics, pre-hospital management, clinical and laboratory parameters, management and outcomes among patients with AKI and without AKI

Baseline characteristics and pre-hospital management are shown in Table 2. Most of the baseline characteristics were similar except patients who lived outside the city (p <0.001), being bitten, in the field (p <0.001), being bitten at the lower extremities (p <0.001), being bitten from snake of the family *Viperidae* or having clinical syndrome of *Viperidae* (p <0.001) were more likely to develop AKI.

Laboratory findings at admission are shown in Table 4However, urine protein to creatinine ratio of patients with different AKI staging was not significantly different (Figure 1).

Fig 1. Spot urine protein to creatinine ratio at admission among patients without acute kidney injury and patients with different stage of acute kidney injury. Data are presented as box and whisker plots with median (horizontal line), interquartile range (box) and maximum value within 1.5 of interquartile range (whiskers).

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Commented [A23]: Minimum age was 12 years to meet study inclusion criteria. So there were only adults in this study? What is the definition of adults? Please clarify this and make revisions throughout the manuscript, if applicable.

Commented [A24]: Here account of only 1 patient with chronic kidney disease is mentioned.
However, in methodology it is written that patients with history of underlying medical illness including neurological diseases, cardiovascular diseases, renal diseases, pulmonary diseases, liver diseases and hematologic diseases were excluded from the study. Please clarify at what stage of patient recruitment, the above exclusion was performed.

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Commented [A26]: Please refer to the table or figure related to these data

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Univariate and multivariate analyses for identifying factors associated with AKI among patients with venomous snakebites

A univariate logistic regression analysis was used to determine which of the baseline characteristics, pre-hospital management, clinical parameters, laboratory findings and management were associated with AKI among patients with venomous snakebites. All clinical factors potentially associated with AKI were included in the univariate logistic regression analysis.

~~All parameters with a p-value ≤0.2 in the univariate logistic regression analysis were then further analyzed by a stepwise multivariate logistic regression analysis using a backward selection method, in order to determine the independent factors significantly associated with AKI.~~ In a multivariate logistic regression model,

Both serum sodium and urine sodium to creatinine ratio were correlated (r = -0.478, p <0.001) (Figure 3a).

Both serum potassium and urine potassium to creatinine ratio were correlated (r = -0.468, p <0.001) (Figure 3b).

Fig 2. Urine electrolytes to creatinine ratio and levels of serum electrolytes among 128 patients with normal kidney function at admission. (A) Urine sodium to creatinine ratio (mmol/mmol) among patients with serum sodium <135 mmol/L and those with serum sodium ≥135 mmol/L. (B) Urine potassium to creatinine ratio (mmol/mmol) among patients with serum potassium <3.5 mmol/L and those with serum potassium ≥3.5 mmol/L. Data are presented as box and whisker plots with median (horizontal line), interquartile range (box) and maximum value within 1.5 of interquartile range (whiskers).

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Commented [A34]: Please show the r and R2 values in the figure 3b.

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Fig 3. Linear regression analysis of urine electrolytes to creatinine ratio on levels of serum electrolytes among 128 patients with normal kidney function at admission. (A) Urine sodium to creatinine ratio (mmol/mmol) on serum sodium (mmol/L) levels among patients with normal renal function. (B) Urine potassium to creatinine ratio (mmol/mmol) on serum potassium (mmol/L) levels among patients with normal renal function.

Commented [A36]: Please show r- values on each figure. It is not statistically meaningful to show correlations that are physiologically not meaningful?

If r-values are the only findings from these figures, it may be worthwhile just providing a narrative of the findings rather than the figures.

Discussion

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Snakebite envenomation was one of the common causes of community-acquired AKI in tropical countries particularly in Southeast Asia [1,2,4]. Renal involvement including proteinuria,

Commented [A38]: These are results. Please do not repeat the results here, only include pertinent interpretation, critical analysis and its discussion.

Of 500 patients with snakebite envenomation, the majority of patients had the bites from Russell's viper (25%) followed by cobra (10%), green pit viper (5%) and sea snake (2%). Previous reports showed that hemotoxic and myotoxic snakes particularly Russell's viper and sea snake are the common cause of renal involvement among patients with snakebite envenomation [7,8]. The incidence of renal involvement with snakebite envenomation was reported in 1.4-28.0% [24,25].Proteinuria >1 g/24 h was observed in 50.0% of patients with Russell's viper bites [26] and hematuria in 35.0% of patents with hemotoxic snakebites or the occurrence of glomerulonephritis after snakebite envenomation [7,8]. Previous reports showed that Russell's viper can cause both intravascular hemolysis and rhabdomyolysis which induced by phospholipase A_2 in snake venom [7,8].

Our multivariate logistic regression analysis showed that the clinical parameters, laboratory findings and the managements which were independently associated with AKI included (1) bites from snake of [redacted]

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Please consider this comment for the whole Discussion section.

[redacted]

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previous reports showed that snakebite related AKI commonly caused from snake of the family

However, our study has some limitations as follows: (1) the majority of patients with venomous snakebites were enrolled from the this is a sample peer review report General Hospital which might cause bias in this study and (2) type of snake could be identified in 67.4% and the rest of 32.6% could not be identified. In case of unidentified snake type, the management of patients would rely on the clinical syndromes of snakebites according to the WHO 2010 guidelines.

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Also a key limitation of the study is that all the data came from three tertiary hospital which severely limits the generalizability of the study findings.

In conclusion, renal manifestations among patients with snake envenomation included reduce urine volume, renal tenderness, proteinuria, hematuria, electrolytes abnormalities and AKI. Snakebite related AKI was a common and significant complication among patients with snake envenomation. The factors associated with snakebite related AKI included bites from snake of the family *Viperidae* or having clinical syndrome of *Viperidae*, duration of bite to needle ≥2 h, presence of hypotension, leukocytosis, overt DIC, rhabdomyolysis and microscopic hematuria. Our findings support the hypothesis of multifactorials involved in the pathogenesis of developing snakebite related AKI. After receiving *Viperidae* bites, a significant number of patients with normal renal function had proximal and distal renal tubular dysfunction. These findings might help clinicians to provide proper management of patients who are at risk for the development of snakebite related AKI in order to reduce the incidence of community-acquired AKI in tropical countries in future.

Competing interests

The authors declare that they have no competing interests.

References

Table 1. Renal manifestations among adults with snakebite envenomation at hospital admission, 2015-2016

Characteristics	[redacted]	[redacted]
Clinical manifestations		
Reduced urine volume	[redacted]	[redacted]
Renal tenderness	[redacted]	[redacted]
Gross hematuria	[redacted]	[redacted]
Dark color urine	[redacted]	[redacted]
Urinalysis and urine chemistries		
Urine protein to creatinine ratio ≥1	[redacted]	[redacted]
Microscopic hematuria	[redacted]	[redacted]
Pigmenturia	[redacted]	[redacted]
Leukocyturia	[redacted]	[redacted]
Blood chemistries		
Acute kidney injury	258	88 (34.1)
Serum sodium		
Hyponatremia	[redacted]	[redacted]6
Hypernatremia	[redacted]	[redacted]
Serum potassium		
Hypokalemia	[redacted]	[redacted]
Hyperkalemia	[redacted]	[redacted]
Serum calcium		
Hypocalcemia	[redacted]	[redacted]
Hypercalcemia	[redacted]	[redacted]

Commented [A41]: Please consider saying clinical and laboratory findings instead of renal findings. This table can be stratified for patients with ARI and no ARI at admission. This table could be combined with table 2.

Commented [A42]: The title needs to reflect the time these parameters were measured.

Commented [A43]: IS it appropriate to place ARI under blood chemistries?

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